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Splicing Misplaced

Newly synthesized transcripts are usually spliced during transcription or immediately thereafter. So pre-mRNA splicing has been presumed to occur exclusively in the cell nucleus. In this issue of *Cell*, Denis et al. (2005) now report the presence of functional spliceosomes and signal-dependent pre-mRNA splicing in the cytoplasm of platelets.

Cellular processes are compartmentalized to increase efficiency and for regulatory purposes. In the case of gene expression, the dogma has been that transcription and pre-mRNA splicing take place in the nucleus, whereas translation occurs in the cytoplasm. However, dogmas exist to be proven wrong. We now know that some transcription also takes place in mitochondria (Gaspari et al., 2004), and it appears that translation can occur in the nucleus (Iborra et al., 2004). In their new study, Denis et al. (2005) add pre-mRNA splicing to the list of misplaced nuclear processes by providing evidence for pre-mRNA splicing in the platelet cytoplasm.

Platelets are highly specialized cells that circulate in the blood and act as first responders to wounds, promoting blood clotting and fighting infection. These cells are generated in the bone marrow by a process of differentiation in which megakaryocytes arrest their proliferation and undergo extensive endoreplication resulting in polyploid cells that are transcriptionally highly active. The megakaryocytes then dramatically expand their cytoplasm, and platelets bud off from specialized membrane regions. The forming platelets contain only cytoplasmic components and have no nucleus (Italiano and Hartwig, 2002; see Figure 1).

Given that anucleate platelets do not contain a genome and therefore do not carry out transcription, there seems to be no need for these cells to have splicing components. Contrary to this expectation, when Denis et al. (2005) analyzed platelets and megakaryocytes, they readily detected many essential pre-mRNA splicing components, including basal splicing factors, serine-arginine-rich (SR) protein splicing factors, and small nuclear splicing RNAs in their cytoplasm. These cytoplasmic components are splicing competent because cellular extracts from platelets support splicing in vitro. Even more remarkably, in addition to splicing factors, resting platelets were found to also contain unspliced pre-mRNAs in their cytoplasm (Denis et al., 2005).

But is the location of splicing factors and pre-mRNAs in platelet cytoplasm of functional relevance? The answer appears to be yes. Denis et al. (2005) provide strong data to suggest that splicing in the cytoplasm is a key regulatory event during platelet activation. One of the pre-mRNAs that they discovered in platelet cytoplasm is that encoding the cytokine interleukin-1β (IL-1β). The presence of this specific transcript is functionally relevant because synthesis of the IL-1β protein is repressed in unstimulated platelets but dramatically increases when platelets are activated. It had been assumed that this regulation involved the activation of mRNA translation. However, Denis et al. (2005) now show that the IL-1β RNA present in the cytoplasm is a partially spliced pre-mRNA. Upon platelet activation, splicing is completed in a constitutive splicing reaction in the activated platelet cytoplasm. This gives rise to an mRNA that can be translated. Thus, pre-mRNA splicing in platelet cytoplasm acts as a key regulatory event in the production of IL-1β during platelet activation.

The scenario that emerges from these findings is one in which during megakaryocyte differentiation, pre-mRNA splicing factors as well as pre-mRNAs destined for platelets are exported into the megakaryocyte cytoplasm and are incorporated into budding platelets (see Figure 1). The splicing factors then may act constitutively on pre-mRNAs or, as in the case of IL-1β, splicing may be triggered upon activation of platelets, serving as a regulatory switch to produce translatable mRNAs.

These results establish two fascinating new paradigms. First, they demonstrate that pre-mRNA splicing is not an exclusively nuclear process. Second, they introduce pre-mRNA splicing as a new regulatory mechanism in the cytoplasm. A precedent for cytoplasmic splicing exists in the unfolded response pathway in yeast, which serves to detect unfolded proteins in the endoplasmic reticulum. However, in that case, the mechanism involves a highly unconventional splicing system consisting of an endonuclease and a tRNA ligase acting on mRNA rather than the new mechanism described in platelets, which relies on the constitutive pre-mRNA splicing machinery (Ruegsegger et al., 2001).

The observations by Denis et al. (2005) raise several fundamental questions regarding the organization and regulatory role of pre-mRNA splicing, and they point to the existence of several unanticipated cellular mechanisms. For one, it is not clear how the splicing machinery normally found in the nucleus ends up in the cytoplasm of megakaryocytes. One possibility is that nuclear components are actively and selectively exported into the megakaryocyte cytoplasm via a specific export mechanism. Alternatively, the redistribution of splicing factors might rely on the intrinsic property of many splicing components to cycle between the nucleus and the cytoplasm as part of their normal life cycle. Many SR protein splicing factors normally undergo nucleocytoplasmic shuttling, and snRNPs are exported from the nucleus during their initial assembly (Cáceres et al., 1998; Yong et al., 2004). Retention of these factors in the megakaryocyte cytoplasm during shuttling would lead to their accumulation outside the nucleus. Regardless of whether accumulation of the splicing machinery in the cytoplasm is caused by increased export or reduced import, this redistribution likely involves new regulatory mechanisms.

An even more puzzling question is how unspliced pre-mRNAs manage to reach the cytoplasm. Incompletely processed pre-mRNAs are usually retained

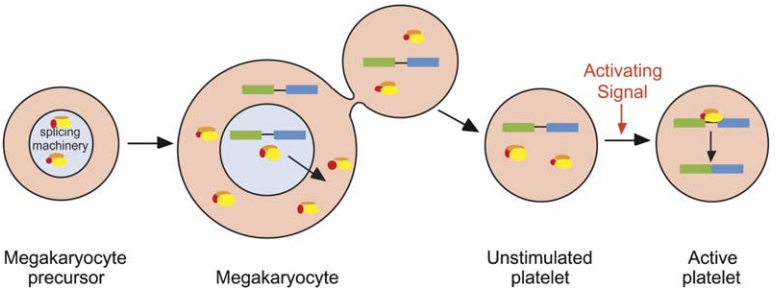

Figure 1. Regulatory Splicing of Pre-mRNA in the Platelet Cytoplasm

Platelets are formed by budding of cytoplasm from megakaryocytes in the bone marrow. Although the splicing machinery is localized in the nucleus of megakaryocyte precursor cells, splicing factors begin to accumulate in the cytoplasm of mature megakaryocytes. The splicing components together with specific partially spliced pre-mRNAs are included in the forming anucleate platelets. Upon stimulation of platelets, the splicing machinery is activated and completes the splicing reaction, giving rise to a translatable mRNA. In this way, cytoplasmic splicing acts as a regulatory mechanism during platelet activation.

within the cell nucleus and rapidly degraded by the exosome as part of the cell's RNA quality-control system (Jensen et al., 2003). It appears that in megakaryocytes, recognition mechanisms must be in place to identify the RNAs destined for platelets and to allow them to evade the RNA degradation machinery, possibly by as-yet-unidentified dedicated RNA export pathways. In the case of IL-1β pre-mRNA, its export might be aided by the partially processed nature of the transcript. Importantly, this type of regulation is not limited to IL-1β, as the authors have already found several additional pre-mRNAs in the platelet cytoplasm.

It is also not clear what signaling events trigger the cytoplasmic splicing reaction upon platelet activation and how they interface with the splicing machinery. The best candidates might be some of the signaling pathways previously implicated in alternative splice-site selection in other cell types (Shin and Manley, 2004). For example, the ERK signaling pathway, which regulates alternative splice-site selection, also has a role in platelet activation and might be a good candidate.

The most provocative question, however, is whether pre-mRNA splicing in the cytoplasm is limited to platelets or whether it might also act on at least some transcripts in cell types that contain a nucleus. Although the levels of splicing factors are generally low in the cytoplasm of most cell types, one should keep in mind that all proteinaceous splicing factors are synthesized in the cytoplasm and that snRNPs are assembled there. If some pre-mRNAs are exported from the nucleus, these splicing components could act in the same was as they do in platelets and support cytoplasmic, regulatory splicing. Although there are no other known examples of splicing in the cytoplasm, not to mention the regulatory function of this process, it is noteworthy that outgrowth of neuronal axons shares many hallmarks characteristic of proplatelet morphogenesis and involves spatially localized posttranscriptional RNA regulation. Admittedly, this is a highly speculative scenario, but in the light of the new work by Denis et al. (2005), we have no choice but to realize that when it comes to splicing, the unexpected must always be expected.

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